Understanding the mechanism which underlies the induction of immunologic tolerance is crucial to the development of strategies for treatment of auto-immune diseases and allograft rejection. Although the concept that T suppressor cells (Ts) downregulate the immune response has long been accepted, the existence of a distinct population of lymphocytes that mediates suppression has not been convincingly demonstrated. In previous studies, human T cell lines (TCLs) were utilized to analyze the suppressive effects of $CD8^+$ $CD28^-$ T cells in allogeneic, peptide specific and xeno-specific responses. In each case, $CD8^+$ $CD28^-$ T cells inhibit proliferation of $CD4^+$ T helper lymphocytes (Th) with cognate antigen specificity. These $CD8^+$ $CD28^-$ T cells display the critical functional characteristics of T suppressor cells. Similar to the induction of $CD8^+$ cytotoxic T cells (Tc) by Th, this process depends on antigen presenting cells (APC) acting as a "bridge" between MHC-class I specific $CD8^+$ and class II specific $CD4^+$ T cells. A possible explanation of Ts-mediated suppression is their ability to modulate the function of APCs. The fourth series of studies herein show that $CD8^+CD28^-$ Ts directly inhibit the CD40 signaling pathway of APC by a contact-dependent mechanism that renders bridging APCs incapable of inducing $CD4^+$ Th activation. The effects of Ts on the functional state of APC supports the concept that the order in which Ts and Th cells interact with cognate APCs determines the functional outcome of immune responses.

On page 112, lines 16-21:

A chimeric peptide tat-DR4, comprising residues 49-57 of HIV-1 tat and residues 64-88 of DRB1*0401 was purchased from Chiron Technologies, Australia. The purity of the peptide

B2 Cont'd

was >85% as determined by reverse-phase HPLC. The amino acid sequence of this peptide is as follows: RKKRRQRRRQKDLLEQKRAAVDTYCRHNYGVGES (SEQ ID NO:382).

On page 130, line 32 through page 131 line 5:

B3

The aim of the present study was to investigate whether the suppressor effect requires the concomitant interaction between Ts, Th and APCs or sequential two cell interactions (first, between Ts and APCs and next, between ''suppressed'' APCs and Th) and whether it is mediated by inhibition of the CD40-signaling pathway.

On page 132, line 20:

B4

Suppression of CD40L Expression on Activated Th Cells

On page 134 line 19 to page 135 line 5:

B5

It is possible that Ts act directly on Th, inhibiting the expression of CD40L or, alternatively, they may act on APCs, blocking the CD40 signaling pathway. To discriminate between these two possibilities, first determined was whether Ts can inhibit Th in the absence of APCs. Experiments in which allospecific Th and Ts were co-cultured in the presence of mAb anti-CD3 showed that Ts do not inhibit Th proliferation or CD40L expression (Figs. 21A, 21B). In contrast, when allospecific Th and Ts are cultured together with the APCs used for priming, both the expression of CD40L and the proliferative capacity of Th are inhibited (Figs. 21C, 21D). These results indicate that the suppressive activity of Ts on Th proliferation is not determined by the direct interaction between Ts and Th and that it requires the presence of APCs. This finding is consistent with the previous observation that Ts and Th must recognize the same APC for suppression to occur [5, 6]. It is, therefore, possible that whether APCs can or cannot activate Th depends

B5 Cont

on their previous encounter with either $CD4^+$ Th or $CD8^+CD28^-$ Ts.

On page 140, line 28 through page 141, line 11:

B6

The data herein support a model in which T-cell mediated suppression can result form the sequential interaction between first, Ts and APCs and next, ''suppressed'' APCs and Th (Fig. 25). In this regard the present findings confirm and extend the ''temporal bridging'' model recently described to account for the complex role that APCs play in Th-mediated generation of $CD8^+$ Tc[2-4]. Furthermore, the present data complement the finding that CD40 signaling is essential for conditioning APCs, by demonstrating that Ts inhibit this pathway. New data show that Ts inhibit Th-induced activation of NF-kB in APC, thus interfering with the upregulation of B7 costimulatory molecules (Li, J., Liu, Z., Jiang, S., Cortesini, R., Lederman, S., Suciu-Foca, N. submitted).

On page 146, lines 2-29:

B7

In the first through fourth series of experiments, we identified and characterized human antigen specific T suppressor cells (Ts). It was shown that Ts inhibits the costimulatory activity of APC blocking NF-κB activation and transcription of costimulatory molecules. To explore the underlying mechanism we used for allostimulating peripheral blood B cells or cells from the dendritic cell line KG-1. Total RNA prepared from KG-1 or from B cells that have been exposed to allospecific Th, Ts or Th/Ts mixtures for 12 hours was used in a cDNA micro-array system to identify genes which are differentially expressed in APC. Although transcription of a wide array of genes was suppressed, expression of 10-15 genes was up-regulated >2-3 fold in APC cocultured for 12 hours with Ts or Ts/Th mixtures. Included in this latter group are the Monocyte Inhibitory Receptor (MIR-10 or ILT4), ILT2 (MIR7), and ILT3. MIR-10, MIR7 (ILT2) and ILT3 belong to a family of leukocyte inhibitory